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Inventor(s) : Richard L. Boyd

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OF THYMIC FUNCTION

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Attached please find the certified copy of the foreign application from which priority is claimed for this case:

Country: PCT
Application No. PCT/AU00/00329
Filing Date: April 17, 2000

In the event any fees are due in connection with the submission of this document, the Commissioner is hereby authorized to charge Deposit Account No. 09-0946.

Dated: August 14, 2003

By:

Respectfully submitted,

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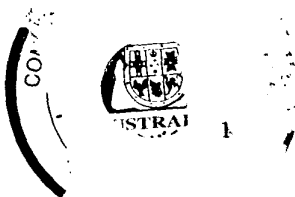
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ALEXANDER JAKSIC
MANAGER
PATENT ADMINISTRATION



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0-5	Petition The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty	
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I	Title of invention	IMPROVEMENT OF T CELL MEDIATED IMMUNITY
II	Applicant	
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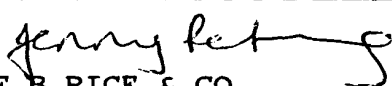
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IV-1	Agent or common representative; or address for correspondence	
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V	Designation of States	
V-1	Regional Patent (other kinds of protection or treatment, if any, are specified between parentheses after the designation(s) concerned)	<p>AP: GH GM KE LS MW SD SL SZ TZ UG ZW and any other State which is a Contracting State of the Harare Protocol and of the PCT</p> <p>EA: AM AZ BY KG KZ MD RU TJ TM and any other State which is a Contracting State of the Eurasian Patent Convention and of the PCT</p> <p>EP: AT BE CH&LI CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE and any other State which is a Contracting State of the European Patent Convention and of the PCT</p> <p>OA: BF BJ CF CG CI CM GA GN GW ML MR NE SN TD TG and any other State which is a member State of OAPI and a Contracting State of the PCT</p>
V-2	National Patent (other kinds of protection or treatment, if any, are specified between parentheses after the designation(s) concerned)	<p>AE AG AL AM AT AU AZ BA BB BG BR BY CA CH&LI CN CR CU CZ DE DK DM DZ EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG US UZ VN YU ZA ZW</p>

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V-5	Precautionary Designation Statement In addition to the designations made under items V-1, V-2 and V-3, the applicant also makes under Rule 4.9(b) all designations which would be permitted under the PCT except any designation(s) of the State(s) indicated under item V-6 below. The applicant declares that those additional designations are subject to confirmation and that any designation which is not confirmed before the expiration of 15 months from the priority date is to be regarded as withdrawn by the applicant at the expiration of that time limit.	
V-6	Exclusion(s) from precautionary designations	NONE
VI-1	Priority claim of earlier national application	
VI-1-1	Filing date	15 April 1999 (15.04.1999)
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VI-2	Priority document request The receiving Office is requested to prepare and transmit to the International Bureau a certified copy of the earlier application(s) identified above as item(s):	VI-1
VII-1	International Searching Authority Chosen	Australian Patent Office (ISA/AU)
VIII	Check list	number of sheets
VIII-1	Request	4
VIII-2	Description	40
VIII-3	Claims	3
VIII-4	Abstract	1
VIII-5	Drawings	22
VIII-7	TOTAL	70
VIII-8	Accompanying items	paper document(s) attached
VIII-8	Fee calculation sheet	✓
VIII-16	PCT-EASY diskette	-
VIII-18	Figure of the drawings which should accompany the abstract	none
VIII-19	Language of filing of the international application	English
IX-1	Signature of applicant or agent	
IX-1-1	Name	F B RICE & CO
IX-1-2	Name of signatory	Jenny Elizabeth Petering

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10-3	Corrected date of actual receipt due to later but timely received papers or drawings completing the purported international application	
10-4	Date of timely receipt of the required corrections under PCT Article 11(2)	
10-5	International Searching Authority	ISA/AU
10-6	Transmittal of search copy delayed until search fee is paid	

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11-1	Date of receipt of the record copy by the International Bureau	
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IMPROVEMENT OF T CELL MEDIATED IMMUNITY

FIELD OF THE INVENTION

5 The present invention concerns methods of modifying the T-cell population make up or increasing the number of T-cells in a subject having depressed or abnormal T-cell population or function. These methods involve disrupting sex steroid signalling to the thymus in the subject.

10 BACKGROUND OF THE INVENTION

The thymus is influenced to a great extent by its bidirectional communication with the neuroendocrine system (Kendall, 1988). Of particular importance is the interplay between the pituitary, adrenals and gonads on thymic function including both trophic (TSH and GH) and
15 atrophic effects (LH, FSH and ACTH) (Kendall, 1988; Homo-Delarche, 1991). Indeed one of the characteristic features of thymic physiology is the progressive decline in structure and function which is commensurate with the increase in circulating sex steroid production around puberty (Hirokawa and Makinodan, 1975; Tosi *et al.*, 1982 and Hirokawa, *et al.*, 1994). The
20 precise target of the hormones and the mechanism by which they induce thymus atrophy is yet to be determined. Since the thymus is the primary site for the production and maintenance of the peripheral T cell pool, this atrophy has been widely postulated as the primary cause of an increased incidence of immune-based disorders in the elderly. In particular,
25 deficiencies of the immune system illustrated by a decrease in T-cell dependent immune functions such as cytolytic T-cell activity and mitogenic responses, are reflected by an increased incidence of immunodeficiency, autoimmunity and tumour load in later life (Hirokawa, 1998).

The impact of thymus atrophy is reflected in the periphery, with
30 reduced thymic input to the T cell pool resulting in a less diverse T cell receptor (TCR) repertoire. Altered cytokine profile (Hobbs *et al.*, 1993; Kurashima *et al.*, 1995); changes in CD4⁺ and CD8⁺ subsets and a bias towards memory as opposed to naive T cells (Mackall *et al.*, 1995) are also observed. Furthermore, the efficiency of thymopoiesis is impaired with age
35 such that the ability of the immune system to regenerate normal T-cell numbers after T-cell depletion, is eventually lost (Mackall *et al.*, 1995).

However, recent work by Douek *et al.* (1998), has shown presumably thymic output to occur even in old age in humans. Excisional DNA products of TCR gene-rearrangement were used to demonstrate circulating, *de novo* produced naive T cells after HIV infection in older patients. The rate of this output and subsequent peripheral T cell pool regeneration needs to be further addressed since patients who have undergone chemotherapy show a greatly reduced rate of regeneration of the T cell pool, particularly CD4⁺ T cells, in post-pubertal patients compared to those who were pre-pubertal (Mackall *et al.*, 1995). This is further exemplified in recent work by Timm and Thoman (1999), who have shown that although CD4⁺ T cells are regenerated in old mice post BMT, they appear to show a bias towards memory cells due to the aged peripheral microenvironment, coupled to poor thymic production of naive T cells.

The thymus essentially consists of developing thymocytes interspersed within the diverse stromal cells (predominantly epithelial cell subsets) which constitute the microenvironment and provide the growth factors and cellular interactions necessary for the optimal development of the T cells. The symbiotic developmental relationship between thymocytes and the epithelial subsets that controls their differentiation and maturation (Boyd *et al.*, 1993), means sex-steroid inhibition could occur at the level of either cell type which would then influence the status of the other. It is less likely that there is an inherent defect within the thymocytes themselves since previous studies, utilising radiation chimeras, have shown that BM stem cells are not affected by age (Hirokawa, 1998; Mackall and Gress, 1997) and have a similar degree of thymus repopulation potential as young BM cells. Furthermore, thymocytes in older aged animals retain their ability to differentiate to at least some degree (Mackall and Gress, 1997; George and Ritter, 1996; Hirokawa *et al.*, 1994). However, recent work by Aspinall (1997), has shown a defect within the precursor CD3⁺CD4⁺CD8⁻ triple negative (TN) population occurring at the stage of TCR β chain gene-rearrangement.

The enormous clinical benefits to be gained through restoration of thymic function, would represent an important strategy for the treatment of immunodeficiencies, particularly in the elderly, HIV patients and following chemotherapy.

SUMMARY OF THE INVENTION

The present inventors have demonstrated that thymic atrophy can be completely reversed by inhibition of sex steroid production, with full restoration of thymic structure and function. The present inventors have also found clinical applications for rejuvenating thymic function by disrupting sex steroid signalling to the thymus.

Accordingly, in a first aspect, the present invention provides a method of modifying the T-cell population makeup or increasing the number of T-cells in a subject having depressed or abnormal T-cell population or function, the method comprising disrupting sex steroid signalling to the thymus in the subject.

Preferably, the modification of T-cell population makeup is characterized by an alteration in the nature and/or ratio of T cell subsets defined functionally and/or by expression of characteristic molecules, wherein the characteristic molecules are selected from the group consisting of: the T cell receptor, CD4, CD8, CD3, CD25, CD28, CD44, CD62L and CD69.

It is further preferred that increasing the number of T-cells in a subject results in a relative increase in T cell numbers when compared to other lymphoid cells. Preferably, the other lymphoid cells are B cells.

It is also preferred that the subject having a depressed or abnormal T-cell population or function is suffering from a condition selected from the group consisting of: cancer, human immunodeficiency virus infection, an autoimmune disease, a hypersensitivity disease or endometriosis.

Preferably, the cancer sufferer has undergone chemotherapy and/or radiation therapy and/or bone marrow transplantation.

Preferably, the subject with the human immunodeficiency virus infection has AIDS.

In a further preferred embodiment, the subject is post-pubertal.

Autoimmune diseases are thought to arise as a polygenic trait, an essential component of which is the participation of pathological self reactive T cells. By treating such subjects with chemotherapy or irradiation, with or without bone marrow transplantation, these self reactive T cells can be ablated. It is envisaged that disruption of sex steroid signalling to the thymus will allow reactivation of the thymus resulting in a cohort of new non-autoreactive T cells.

Accordingly, in a second aspect the present invention provides a method for treating an autoimmune disease in a subject, the method comprising the steps; ablating the resident T cell population, and disrupting sex steroid signalling to the thymus in the subject.

5 The steps of the second aspect of the present invention can be performed in any order.

In a preferred embodiment, this method further comprises subjecting the individual to a bone marrow transplant.

10 In a further preferred embodiment, the T cell population is ablated by exposing the individual to chemotherapy or irradiation.

The present invention may also be utilized to enhance an immune response to an antigen in a subject.

15 Accordingly, in a third aspect the present invention provides a method for enhancing an immune response to an antigen in a subject, the method comprising disrupting sex steroid signalling to the thymus in the subject, and administering an antigen.

The antigen may be, for example, derived from an infectious agent(s) or from a tumour cell.

20 In a preferred embodiment of the third aspect, the subject is suffering from cancer or an infection.

In a further preferred embodiment of the third aspect, the antigen is mixed with an adjuvant before administration.

25 In a fourth aspect, the present invention provides a method of decreasing host-vs-graft reaction in a subject following transplantation of an organ, the method comprising the following steps:

ablating T-cells in the subject;

disrupting sex steroid signalling to the thymus in the subject; and transplanting an organ from a donor into the subject.

30 Preferably, the method of the fourth aspect also comprises transplanting bone marrow to the subject from the donor.

With respect to each of the methods of the present invention, it is preferred that sex steroid signalling to the thymus is disrupted by inhibiting sex steroid production or by blocking a sex steroid receptor(s) within the thymus.

35 Preferably, inhibition of sex steroid production is achieved by either castration or administration of a sex steroid analogue(s).

Preferred sex steroid analogues include, eulexin, goserelin, leuprolide, dioxalan derivatives such as triptorelin, meterelin, buserelin, histrelin, nafarelin, lutrelin, leuprorelin, and luteinizing hormone-releasing hormone analogues.

5 Currently, it is preferred that sex steroid analogue is an analogue of luteinizing hormone-releasing hormone. More preferably, the luteinizing hormone-releasing hormone analogue is deslorelin.

10 In yet another preferred embodiment, the sex steroid analogue(s) is administered by a sustained peptide-release formulation. Preferred sustained peptide-release formulations are provided in WO 98/08533, the entire contents of which are incorporated herein by reference.

15 In a fifth aspect, the present invention provides a composition for enhancing an immune response to an antigen in a subject, the composition comprising an adjuvant, the antigen, and an analogue of luteinizing hormone-releasing hormone.

20 It will also be understood by the skilled addressee that the present invention can be applied to any organism which possesses a thymus at some stage during its development. Preferably, the organism is a mammal. More preferably, the organism is a human.

25 Throughout this specification the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element, integer or step, or group of elements, integers or steps, but not the exclusion of any other element, integer or step, or group of elements, integers or steps.

BRIEF DESCRIPTION OF THE ACCOMPANYING FIGURES

30 **Fig. 1:** Changes in thymocyte number pre- and post-castration. Thymus atrophy results in a significant decrease in thymocyte numbers with age. By 2 weeks post- castration, cell numbers have increased to young adult levels. By 3 weeks post-castration, numbers have significantly increased from the young adult and they are stabilised by 4 weeks post-castration. ***=Significantly different from young adult (2 mth) thymus, $p < 0.001$

35 **Fig. 2:** (A) Spleen numbers remain constant with age and post-castration. The B:T cell ratio in the periphery also remains constant (B),

however, the CD4:CD8 ratio decreases significantly ($p < 0.001$) with age and is restored to normal young levels by 4 weeks post-ex.

5 **Fig. 3:** FACS profiles of CD4 vs. CD8 thymocyte populations with age and post-castration. Percentages for each quadrant are given above each plot. Subpopulations of thymocytes remain constant with age and there is a synchronous expansion of thymocytes following castration.

10 **Fig. 4.1:** Proliferation of thymocytes as detected by incorporation of a pulse of BrdU. Proportion of proliferating thymocytes remains constant with age and following castration.

15 **Fig. 4.2:** Effects of age and castration on proliferation of thymocyte subsets. (A) Proportion of each subset that constitutes the total proliferating population. The proportion of CD8+ T cells within the proliferating population is significantly increased. (B) Percentage of each subpopulation that is proliferating. The TN and CD8 Subsets have significantly less proliferation at 2 years than at 2 months. At 2 weeks post-castration, the TN population has returned to normal young levels of proliferation while the
20 CD8 population shows a significant increase in proliferation. The level is equivalent to the normal young by 4 weeks post-castration. (C) Overall TN proliferation remains constant with age and post-castration, however, the significant decrease in proliferation of the TN1 subpopulation with age, is not returned to normal levels by 4 weeks post-castration (D).***=Highly
25 significant, $p < 0.001$, **=significant, $p < 0.01$

30 **Fig. 5:** Migration rates from 1 year and 2 year mice as determined by IT FITC labelling. Young adult migration rates are 1% per day. Controls used were non-injected animals. Migration rates remain constant with age.

35 **Fig. 6:** Changes in thymus, spleen and lymph node cell numbers following treatment with cyclophosphamide, a chemotherapy agent. Note the rapid expansion of the thymus in castrated animals when compared to the non-castrate (cyclo alone) group at 1 and 2 weeks post-treatment. In addition, spleen and lymph node numbers of the castrate group were well

increased compared to the cyclophosphamide alone group. By 4 weeks, cell numbers are normalised. (n = 3-4 per treatment group and time point).

5 **Fig. 7:** Changes in thymus, spleen and lymph node cell numbers following irradiation (625 Rads). Note the rapid expansion of the thymus in castrated animals when compared to the non-castrate (irradiation alone) group at 1 and 2 weeks post-treatment. By 4 weeks, cell numbers are normalised. (n = 3-4 per treatment group and time point).

10 **Fig. 8:** Changes in thymus, spleen and lymph node cell numbers following irradiation. Note the rapid expansion of the thymus in castrated animals when compared to the non-castrate group at 1 and 2 weeks post-treatment. However, the difference observed is not as obvious as when mice were castrated 1 week prior to treatment (Fig. 8). By 4 weeks, cell numbers
15 are normalised. (n = 3-4 per treatment group and time point).

Fig. 9: Changes in thymus, spleen and lymph node cell numbers following treatment with cyclophosphamide, a chemotherapy agent. Note the rapid expansion of the thymus in castrated animals when compared to
20 the non-castrate (cyclophosphamide alone) group at 1 and 2 weeks post-treatment. In addition, spleen and lymph node numbers of the castrate group were well increased compared to the cyclophosphamide alone group. By 4 weeks, cell numbers are normalised. (n = 3-4 per treatment group and time point). Chemical castration is comparable to surgical castration in
25 regeneration of the immune system post-cyclophosphamide treatment.

Fig. 10: Lymph node cellularity following foot-pad immunisation with HSV-1. Note the increased cellularity in the aged post-castration as compared to the non-castrated group. Bottom graph illustrates the overall
30 activated cell number as gated on CD25 vs. CD8 cells by FACS.

Fig. 11: Examples of Flow cytometry dot plots illustrating activated cell proportions in lymph nodes following HSV-1 immunisation. Activated cells are CD25/CD8 double-positive.
35

Fig. 12: V β 10 expression on CTL in activated LN following HSV-1 inoculation. Note the diminution of a clonal response in aged mice and the reinstatement of the expected response post-castration.

5 **Fig. 13:** Changes in thymus, spleen, lymph node and bone marrow cell numbers following bone marrow transplantation of Ly5 congenic mice. Note the rapid expansion of the thymus in castrated animals when compared to the non-castrate group at all time points post-treatment. In addition, spleen and lymph node numbers of the castrate group were well increased compared to the cyclophosphamide alone group. (n = 3-4 per treatment group and time point). Castrated mice had significantly increased congenic (Ly5.2) cells compared to non-castrated animals (data not shown).

15 **Fig. 14:** Changes in thymus cell number in castrated and noncastrated mice after foetal liver reconstitution. n = 3-4 for each test group. (A) At two weeks, thymus cell number of castrated mice was at normal levels and significantly higher than that of noncastrated mice (*p \leq 0.05). Hypertrophy was observed in thymii of castrated mice after four weeks. Noncastrated cell numbers remain below control levels. (B) CD45.2⁺ cells-CD45.2⁺ is a marker showing donor derivation. Two weeks after reconstitution donor-derived cells were present in both castrated and noncastrated mice. Four weeks after treatment approximately 85% of cells in the castrated thymus were donor-derived. There were no donor-derived cells in the noncastrated thymus.

25 **Fig. 15:** FACS profiles of CD4 versus CD8 donor derived thymocyte populations after lethal irradiation and foetal liver reconstitution. Percentages for each quadrant are given to the right of each plot. The age matched control profile is of an eight month old Ly5.1 congenic mouse thymus. Those of castrated and noncastrated mice are gated on CD45.2⁺ cells, showing only donor derived cells. Two weeks after reconstitution subpopulations of thymocytes do not differ between castrated and noncastrated mice.

35 **Fig. 16:** Myeloid and lymphoid dendritic cell (DC) number after lethal irradiation, foetal liver reconstitution and castration. n= 3-4 mice for each test group. Control (white) bars on the following graphs are based on the

normal number of dendritic cells found in untreated age matched mice. (A) Donor-derived myeloid dendritic cells-Two weeks after reconstitution DC were present at normal levels in noncastrated mice. There were significantly more DC in castrated mice at the same time point. (* $p \leq 0.05$). At four weeks DC number remained above control levels in castrated mice. (B) Donor-derived lymphoid dendritic cells - Two weeks after reconstitution DC numbers in castrated mice were double those of noncastrated mice. Four weeks after treatment DC numbers remained above control levels.

Fig. 17: Changes in total and CD45.2⁺ bone marrow cell numbers in castrated and noncastrated mice after foetal liver reconstitution. $n=3-4$ mice for each test group. (A) Total cell number - Two weeks after reconstitution bone marrow cell numbers had normalised and there was no significant difference in cell number between castrated and noncastrated mice. Four weeks after reconstitution there was a significant difference in cell number between castrated and noncastrated mice (* $p \leq 0.05$). (B) CD45.2⁺ cell number - There was no significant difference between castrated and noncastrated mice with respect to CD45.2⁺ cell number in the bone marrow, two weeks after reconstitution. CD45.2⁺ cell number remained high in castrated mice at four weeks. There were no donor-derived cells in the noncastrated mice at the same time point.

Fig. 18: Changes in T cells and myeloid and lymphoid derived dendritic cells (DC) in the bone marrow after foetal liver reconstitution. $n=3-4$ mice for each test group. Control (white bars on the following graphs are based on the normal number of T cells and dendritic cells found in untreated age matched mice. (A) T cell number - Numbers were reduced two and four weeks after reconstitution in both castrated and noncastrated mice. (B) Donor derived myeloid dendritic cells - Two weeks after reconstitution DC cell numbers were normal in both castrated and noncastrated mice. At this time point there was no significant difference between numbers in castrated and noncastrated mice. (C) Donor-derived lymphoid dendritic cells - Numbers were at normal levels two and four weeks after reconstitution. At two weeks there was no significant difference between numbers in castrated and noncastrated mice.

Fig. 19: Change in total and CD45.2⁺ spleen cell numbers in castrated and noncastrated mice after foetal liver reconstitution. n=3-4 mice for each test group. (A) Total cell number - Two weeks after reconstitution cell numbers were decreased and there was no significant difference in cell number between castrated and noncastrated mice. Four weeks after reconstitution cell numbers were approaching normal levels in castrated mice. (B) CD45.2⁺ cell number- there was no significant difference between castrated and noncastrated mice with respect to CD45.2⁺ cell number in the spleen, two weeks after reconstitution. CD45.2⁺ cell number remained high in castrated mice at four weeks. There were no donor-derived cells in the noncastrated mice at the same time point.

Fig. 20: Splenic changes in T cells and myeloid and lymphoid derived dendritic cells (DC) after foetal liver reconstitution. n=3-4 mice for each test group. Control (white) bars on the following graphs are based on the normal number of T cells and dendritic cells found in untreated age matched mice. (A) T cell number - Numbers were reduced two and four weeks after reconstitution in both castrated and noncastrated mice. (B) Donor derived myeloid dendritic cells - two and four weeks after reconstitution DC numbers were normal in both castrated and noncastrated mice. At two weeks there was no significant difference between numbers in castrated and noncastrated mice. (C) Donor-derived lymphoid dendritic cells - numbers were at normal levels two and four weeks after reconstitution. At two weeks there was no significant difference between numbers in castrated and noncastrated mice.

Fig. 21: Changes in total and CD45.2⁺ lymph node cell numbers in castrated and noncastrated mice after foetal liver reconstitution. n=3-4 for each test group. (A) Total cell numbers - two weeks after reconstitution cell numbers were at normal levels and there was no significant difference in cell number between castrated and noncastrated mice. Four weeks after reconstitution cell numbers in castrated mice were at normal levels. (B) CD45.2⁺ cell number - There was no significant difference between castrated and noncastrated mice with respect to CD45.2⁺ cell number in the lymph node, two weeks after reconstitution. CD45.2 cell number remained high in castrated mice at four weeks. There were no donor-derived cells in the noncastrated mice at the same point.

Fig. 22: Changes in T cells and myeloid and lymphoid derived dendritic cells (DC) in the mesenteric lymph nodes after foetal liver reconstitution. n=3-4 mice for each test group. Control (white) bars on the following graphs are based on the normal number of T cells and dendritic cells found in untreated age matched mice. (A) T cell number - Numbers were reduced two and four weeks after reconstitution in both castrated and noncastrated mice. (B) Donor derived myeloid dendritic cells - Two weeks after reconstitution DC numbers were normal in both castrated and noncastrated mice. At four weeks they were decreased. At two weeks there was no significant difference between numbers in castrated and noncastrated mice. (C) Donor-derived lymphoid dendritic cells - numbers were at normal levels two and four weeks after reconstitution. At two weeks there was no significant difference between numbers in castrated and noncastrated mice.

DETAILED DESCRIPTION OF THE INVENTION

The phrase "modifying the T-cell population makeup" refers to altering the nature and/or ratio of T cell subsets defined functionally and by expression of characteristic molecules. Examples of these characteristic molecules include, but are not limited to, the T cell receptor, CD4, CD8, CD3, CD25, CD28, CD44, CD62L and CD69.

The phrase "increasing the number of T-cells" refers to an absolute increase in the number of T cells in a subject in the thymus and/or in circulation and/or in the spleen and/or in the bone marrow and/or in peripheral tissues such as lymph nodes, gastrointestinal, urogenital and respiratory tracts. This phrase also refers to a relative increase in T cells, for instance when compared to B cells.

A "subject having a depressed or abnormal T-cell population or function" includes an individual suffering from cancer, especially one who has undergone chemotherapy or radiation therapy, or has been subjected to a bone marrow transplant, or breast and prostate cancer patients, or any cancer or proliferative disorder resulting in T cell abnormalities or reduced functional capacity of cell-mediated immunity. This phrase also includes an individual infected with the human immunodeficiency virus, especially one who has AIDS. Furthermore, this phrase includes any post-pubertal